OIPE JC78
SEP 06 2002
PATENT & TRADEMARK OFFICE

RECEIVED
SEP 11 2002
TECH CENTER 1600/2900

PATENT
09

#14
9-12-02

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT(S): Kalle SAKSELA et al.

APPLICATION NO.: 09/579,894 GROUP: 1627

FILED: May 26, 2000 EXAMINER: B. Celsa

FOR: METHODS AND MATERIALS FOR GENERATING SH3 DOMAINS WITH TAILORED BINDING PROPERTIES

DECLARATION SUBMITTED UNDER 37 C.F.R.§1.132

Honorable Commissioner of Patents
Washington, D.C. 20231

I, Dr. Kalle SAKSELA, do hereby declare the following.

I am a co-inventor of the subject matter disclosed in the above-captioned application.

I am also the responsible (i.e. corresponding) author on the article Lee et al. *The EMBO J.* 14 5006-5015 (1995), which the Examiner cites in the Office Action of May 6, 2002.

As such, I am singularly qualified to discuss the present invention and the work that was done in Lee et al.

I have further reviewed the Office Action of May 6, 2002 and the Examiner's interpretation of the Lee et al. reference.

In my opinion, the Examiner has misinterpreted the work that we report in Lee et al. Lee et al. neither

discloses nor suggests the work later done with the present invention.

The Examiner asserts that Lee et al. is not limited to the Fyn SH3 RT-loop and extends to multiple SH3 kinase domains, which contain multiple mutant RT-loops. The Examiner further interprets Lee et al. as teaching and/or suggesting making artificial SH3 domains of different kinases that contain random substitutions within the non-conserved regions of the RT-loop region. However, the Examiner has completely misinterpreted the work we reported in Lee et al.

In the Lee et al. article, we showed that the RT loop of SH3 is important with respect to binding specificity. Namely, when one, two or three amino acids (most notably the Ile residue 96) identical or similar to those found in the Hck tyrosine kinase, were transferred into the corresponding position in the RT-loop of the Fyn tyrosine kinase, the binding properties of Fyn (i.e. ability to bind to HIV Nef) could be made to resemble those of Hck.

With the work done in Lee et al., we did not create totally new binding properties, rather we merely mimicked what already exists in nature. In other words, when Fyn was made structurally similar to Hck, its function, i.e. binding properties became that of Hck. Thus, the important

and sole teaching of Lee et al. is that the more closely the amino acid sequence in the RT-loop of one SH3 domain is made to resemble that of another SH3 domain, the more similar will their binding specificities become. It is critical to note that Lee et al. did not suggest or even hint that an artificial SH3 domain able to bind to HIV Nef could be made by any other way than by mimicking the RT-loop sequence of the Hck tyrosine kinase. In fact, based on the teachings of Lee at al. this would seem unlikely.

By contrast, the present invention discloses that specific binding to HIV Nef can be achieved by combinations of six RT-loop amino acids that bear no similarity whatsoever with the corresponding sequence in Hck or in any other known natural SH3 domain.

In other words, the amino acid sequences of the RT loops of the artificial SH3 domains as produced in the present invention, which sequences bind strongly to HIV-1 Nef protein, do not reflect any naturally occurring amino acid sequences and therefore cannot be achieved on the basis of the existing prior art, including the work we did in Lee et al. It should be noted that in nature only a limited number of SH3 domains exist (the human genome appears to encode about 200 different domains).

Thus by mimicking nature, as we did in Lee et al., the amount of naturally occurring domains is the maximum limit of different binding specificities that may be obtained. On the other hand, by using an artificial RRT-SH3 library of the present invention, we have about achieved 64 million different SH3 binding domains, and thus the present invention provides an almost unlimited source of new binding properties.

Another key aspect and advantage of the invention is that one can obtain unnaturally high binding affinities as measured by the strength of binding, which is never possible by mimicking nature, as with Lee et al. With the present invention, artificial SH3 domains can be produced which can displace the natural SH3-mediated protein interactions, which will be useful in both experimental and therapeutic applications. The ability to obtain extremely high binding affinities is not possible from Lee et al.

The Examiner is of the opinion that modification of the Fyn-SH3 to mimic Hck is a "general example" and that based on that example many different SH3 domains could be modified in various ways. However, in Lee et al. we only described how the binding capacity of one known SH3 domain can be changed to the binding capacity of another known SH3 domain. Consequently, although the FynR961 of Lee et al.

is "artificial" in the sense that the protein is different from wild-type Fyn-SH3, the isoleucine at this position is copied directly from nature. Thus, while FynR961 and the other Hck-like Fyn-SH3 variants may be termed "artificial", they are not, in fact, artificial in the sense that they were engineered to resemble a natural molecule, i.e. Hck. We in no way disclosed in the Lee et al. article any modification of Hck or any other SH3 domain other than by modifying the Hck to mimic other known, naturally occurring SH3 domains.

The Examiner also is of the position that in Lee et al. we described the preparation of an artificial SH3 domain that can have "random" amino acids. However, the Examiner has misinterpreted the reference. There is no disclosure of randomization in Lee et al. "Randomization" means the creation of totally new peptides having one or more amino acid substitutions. With the work in Lee et al. we merely described a study of how Fyn and Hck are different from each other with regard their respective binding to Nef protein and how Fyn can be deliberately, i.e. not randomly, improved by using Hck as a model.

The Examiner states that we used recombinant libraries. However, the Examiner has again misinterpreted the reference. In Lee et al., we used a small number of

recombinant plasmids, which encoded proteins whose ability to bind Nef protein had been tested, one at a time. The set of plasmids used in Lee et al. is not a "library" nor would it considered a "library" by one skilled in the art. A "library" is made of a great number of recombinant vectors, from which the best or most appropriate is selected. The present invention exploits the SH3 library created by the inventors. In Lee et al. we did not use a library nor randomization. The designed and specifically directed mutagenesis of Lee et al. is an approach that is completely the opposite of the randomized library of the invention.

With Lee et al., we deliberately used targeted mutagenesis to specifically change amino acids to copy the SH3 domains of other naturally occurring proteins. With the present invention we used randomized libraries to create completely new SH3 domains with new binding properties. As such, in my opinion we did not achieve or suggest the present invention with the work we describe in Lee et al.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge

that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Kalle SAKSELA

SEPT. 6, 2002
Date